● PRINTER RUSH ●

(PTO ASSISTANCE)

FBW

Application :	09/833118	**Examiner :**	Carlson	**GAU :**	1653
From:	Tw	**Location:**	IDC FMF (FDC)	**Date:**	4-17-01

Tracking #: 59N932 Week Date: 3-8-04

DOC CODE	DOC DATE	MISCELLANEOUS
☐ 1449		☐ Continuing Data
☐ IDS		☐ Foreign Priority
☐ CLM		☐ Document Legibility
☐ IIFW		☐ Fees
☐ SRFW		☐ Other
☐ DRW		
☐ OATH		
☐ 312		
☒ SPEC	4-12-01	

[RUSH] **MESSAGE:**

In the specification submitted on 4-12-01 there is missing data at Lines 25 and 26 of Page 77.

Please supply data

Thank You

Tw

[XRUSH] **RESPONSE:** Corrected

See Attachment

INITIALS: KP

NOTE: This form will be included as part of the official USPTO record, with the Response document coded as XRUSH.

REV 10/04

entirety by reference herein). Studies revealed that the Hsp150 protein is inefficiently separated from rHA by ion exchange chromatography. The mutation in the HSP150 gene removes a potential contaminant that has proven difficult to remove by standard purification techniques. See, e.g., U.S. Patent No. 5,783,423, hereby incorporated in its entirety by reference herein.

The desired protein is produced in conventional ways, for example from a coding sequence inserted in the host chromosome or on a free plasmid. The yeasts are transformed with a coding sequence for the desired protein in any of the usual ways, for example electroporation. Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente (1990) *Methods Enzymol.* 194, 182.

Successfully transformed cells, *i.e.*, cells that contain a DNA construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct can be grown to produce the desired polypeptide. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern (1975) *J. Mol. Biol.* 98, 503 or Berent *et al.* (1985) *Biotech.* 3, 208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies.

Useful yeast plasmid vectors include pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers HIS3, 7RP1, LEU2 and URA3. Plasmids pRS413-416 are Yeast Centromere plasmids (Ycps).

Preferred vectors for making albumin fusion proteins for expression in yeast include pPPC0005, pScCHSA, pScNHSA, and pC4:HSA which are described in detail in Example 2. Figure 4 shows a map of the pPPC0005 plasmid that can be used as the base vector into which polynucleotides encoding Therapeutic proteins may be cloned to form HA-fusions. It contains a *PRB1 S. cerevisiae* promoter (PRB1p), a Fusion leader sequence (FL), DNA encoding HA (rHA) and an *ADH1 S. cerevisiae* terminator sequence. The sequence of the fusion leader sequence consists of the first 19 amino acids of the signal peptide of human serum albumin (SEQ ID NO:29) and the last five amino acids of the mating factor alpha 1 promoter (SLDKR, see EP-A-387 319 which is hereby incorporated by reference in its entirety.

The plasmids, pPPC0005, pScCHSA, pScNHSA, and pC4:HSA were deposited on April 11, 2001 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, ~~and given accession numbers ATCC ____, ____, ____ and ____, respectively.~~ Another vector useful for expressing an albumin fusion protein in

5.10.05